FOCUSED LIBRARIES OF GENETIC PACKAGES

This application claims the benefit under 35 USC § 120 of United States provisional application 60/256,380, filed December 18, 2001. The provisional application and the Tables attached to it are specifically incorporated by reference herein.

The present invention relates to focused libraries of genetic packages that each display, display and express, or comprise a member of a diverse family of peptides, polypeptides or proteins and collectively display, display and express, or comprise at least a portion of the focused diversity of the family. The focused diversity of the libraries of this invention comprises both sequence diversity and length diversity. In a preferred embodiment, the focused diversity of the libraries of this invention is biased toward the natural diversity of the selected family. In a more preferred embodiment, the libraries are biased toward the natural diversity of human antibodies and are characterized by variegation in their heavy chain and light chain complementarity determining regions ("CDRs").

The present invention further relates to vectors and genetic packages (e.g., cells, spores or viruses) for displaying, or displaying and expressing a focused diverse family of peptides, polypeptides or proteins. In a

preferred embodiment the genetic packages are filamentous phage or phagemids or yeast. Again, the focused diversity of the family comprises diversity in sequence and diversity in length.

The present invention further relates to methods of screening the focused libraries of the invention and to the peptides, polypeptides and proteins identified by such screening.

BACKGROUND OF THE INVENTION

It is now common practice in the art to prepare libraries of genetic packages that individually display, display and express, or comprise a member of a diverse family of peptides, polypeptides or proteins and collectively display, display and express, or comprise at least a portion of the amino acid diversity of the family. In many common libraries, the peptides, polypeptides or proteins are related to antibodies (e.g., single chain Fv (scFv), Fv, Fab, whole antibodies or minibodies (i.e., dimers that consist of V_H linked to V_L)). Often, they comprise one or more of the CDRs and framework regions of the heavy and light chains of human antibodies.

Peptide, polypeptide or protein libraries have been produced in several ways in the prior art. See e.g., Knappik et al., J. Mol. Biol., 296, pp. 57-86 (2000), which is incorporated herein by references. One method is to capture the diversity of native donors, either naive or immunized. Another way is to generate libraries having synthetic diversity. A third method is a combination of the first two. Typically, the diversity produced by these methods is limited to sequence diversity, i.e., each member of the library differs from the other members of the family by having different amino acids or variegation at a given

position in the peptide, polypeptide or protein chain. Naturally diverse peptides, polypeptides or proteins, however, are not limited to diversity only in their amino acid sequences. For example, human antibodies are not limited to sequence diversity in their amino acids, they are also diverse in the lengths of their amino acid chains.

For antibodies, diversity in length occurs, for example, during variable region rearrangements. See e.g., Corbett et al., J. Mol. Biol., 270, pp. 587-97 (1997). The joining of V genes to J genes, for example, results in the inclusion of a recognizable D segment in CDR3 in about half of the heavy chain antibody sequences, thus creating regions encoding varying lengths of amino acids. The following also may occur during joining of antibody gene segments: (i) the end of the V gene may have zero to several bases deleted or changed; (ii) the end of the D segment may have zero to many bases removed or changed; (iii) a number of random bases may be inserted between V and D or between D and J; and (iv) the 5' end of J may be edited to remove or to change several bases. These rearrangements result in antibodies that are diverse both in amino acid sequence and in length.

Libraries that contain only amino acid sequence diversity are, thus, disadvantaged in that they do not reflect the natural diversity of the peptide, polypeptide or protein that the library is intended to mimic. Further, diversity in length may be important to the ultimate functioning of the protein, peptide or polypeptide. For example, with regard to a library comprising antibody regions, many of the peptides, polypeptides, proteins displayed, displayed and expressed, or comprised by the genetic packages of the library may not fold properly or their binding to an antigen may be disadvantaged, if

diversity both in sequence and length are not represented in the library.

An additional disadvantage of prior art libraries of genetic packages that display, display and express, or comprise peptides, polypeptides and proteins is that they are not focused on those members that are based on natural occurring diversity and thus on members that are most likely to be functional. Rather, the prior art libraries, typically, attempt to include as much diversity or variegation at every amino acid residue as possible. This makes library construction time-consuming and less efficient than possible. The large number of members that are produced by trying to capture complete diversity also makes screening more cumbersome than it needs to be. This is particularly true given that many members of the library will not be functional.

SUMMARY OF THE INVENTION

One objective of this invention is focused libraries of vectors or genetic packages that encode members of a diverse family of peptides, polypeptides or proteins wherein the libraries encode populations that are diverse in both length and sequence. The diverse length comprising components that contain motifs that are likely to fold and function in the context of the parental peptide, polypeptide or protein.

Another object of this invention is focused libraries of genetic packages that display, display and express, or comprise a member of a diverse family of peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the focused diversity of the family. These libraries are diverse not only in their amino acid

sequences, but also in their lengths. And, their diversity is focused so as to more closely mimic or take into account the naturally-occurring diversity of the specific family that the library represents.

Another object of this invention is diverse, but focused, populations of DNA sequences encoding peptides, polypeptides or proteins suitable for display or display and expression using genetic packages (such as phage or phagemids) or other regimens that allow selection of specific binding components of a library.

A further object of this invention is focused libraries comprising the CDRs of human antibodies that are diverse in both their amino acid sequence and in their length (examples of such libraries include libraries of single chain Fv (scFv), Fv, Fab, whole antibodies or minibodies (i.e., dimers that consist of V_H linked to V_L)). Such regions may be from the heavy or light chains or both and may include one or more of the CDRs of those chains. More preferably, the diversity or variegation occurs in all of the heavy chain and light chain CDRs.

It is another object of this invention to provide methods of making and screening the above libraries and the peptides, polypeptides and proteins obtained in such screening.

Among the preferred embodiments of this invention are the following:

1. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by

variegated DNA sequences that encode a heavy chain CDR1 selected from the group consisting of:

(1) $<1>_1Y_2<1>_3M_4<1>_5$, wherein <1> is an equimolar mixture of each of amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;

(2) $(S/T)_1(S/G/X)_2(S/G/X)_3Y_4Y_5W_6(S/G/X)_7$. wherein (S/T) is a 1:1 mixture of S and T residues, (S/G/X) is a mixture of 0.2025 S, 0.2025 G and 0.035 of each of amino acid residues A, D, E, F, H, I, K, L, M, N, P, Q, R, T, V, W, and Y;

(3) $V_1S_2G_3G_4S_5I_6S_7<1>_8<1>_9<1>_{10}Y_{11}Y_{12}W_{13}<1>_{14}$, wherein <1> is an equimolar mixture of each of amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y; and

(4) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio: HC CDR1s (1):(2):(3)::0.80:0.17:0.02.

2. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody facility, the vectors or genetic packages being characterized by variegated DNA sequences that encode a heavy chain CDR2 selected from the group consisting of:

(1) <2>I<2><3>SGG<1>T<1>YADSVKG, wherein <1> is an equimolar mixture of each of amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y; <2> is an equimolar mixture of each of amino acid residues Y, R, W, V, G, and S; and <3> is an equimolar

mixture of each of amino acid residues P, S, and G or an equimolar mixture of P and S;

(2) <1>I<4><1><1><G><5><1><1><1>YADSVKG, wherein <1> is an equimolar mixture of each of amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y; <4> is an equimolar mixture of residues D, I, N, S, W, Y; and <5> is an equimolar mixture of residues S, G, D and N;

(3) <1>I<4><1><1>G<5><1><1>YNPSLKG, wherein <1> is an equimolar mixture of each of amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and <4> and <5> are as defined above;

(4) <1>I<8>S<1><1><1>GGYY<1>YAASVKG, wherein <1> is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; <8> is 0.27 R and 0.027 of each of ADEFGHIKLMNPQSTVWY; and

(5) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio: HC CDR2s: (1)/(2) (equimolar): (3):(4)::0.54:0.43:0.03.

3. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by variegated DNA sequences that encode a heavy chain CDR3 selected from the group consisting of:

(1) YYCA21111YFDYWG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F,

G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(2) YYCA2111111YFDYWG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(3) YYCA211111111YFDAYTG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(4) YYCAR111S2S3111YFDYWG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of S and G; and 3 is an equimolar mixture of Y and W;

(5) YYCA2111CSG11CY1YFDYWG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(6) YYCA211S1TIFG11111YFDYWG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; and 2 is an equimolar mixture of K and R;

(7) YYCAR111YY2S3344111YFDYWG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; 2 is an equimolar mixture of D and S; and 3 is an equimolar mixture of S and G;

(8) YYCAR1111YC2231CY111YFDYWG, wherein 1 is an equimolar mixture of each amino acid residues A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y; 2 is an equimolar mixture of S and G; and 3 is an equimolar mixture of T, D and G; and

(9) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably the HC CDR3s (1) through (8) are in the following proportions in the mixture:

(1) 0.10
(2) 0.14
(3) 0.25
(4) 0.13
(5) 0.13
(6) 0.11
(7) 0.04 and
(8) 0.10; and more preferably the HC CDR3s (1) through (8) are in the following proportions in the mixture:

(1) 0.02
(2) 0.14
(3) 0.25
(4) 0.14
(5) 0.14
(6) 0.12
(7) 0.08 and
(8) 0.11.

Preferably, 1 in one or all of HC CDR3s (1) through (8) is 0.095 of each of G and Y and 0.048 of each of A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, and W.

4. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by

variegated DNA sequences that encodes a kappa light chain CDR1 selected from the group consisting of:

(1) RASQ<1>V<2><2><3>LA

(2) RASQ<1>V<2><2><2><3>LA;

wherein <1> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY; <2> is 0.2 S and 0.044 of each of ADEFGHIKLMNPQRTVWY; and <3> is 0.2Y and 0.044 each of ADEFGHIKLMNPQRTVW and Y; and

(3) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio CDR1s (1):(2)::0.68:0.32.

5. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by variegated DNA sequences that encode a kappa light chain CDR2 having the sequence:

<1>AS<2>R<4><1>,

wherein <1> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY; <2> is 0.2 S and 0.044 of each of ADEFGHIKLMNPQRTVWY; and <4> is 0.2 A and)0.044 each of DEFGHIKLMNPQRSTVWY.

6. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the

vectors or genetic packages being characterized by variegated DNA sequences that encode a kappa light chain CDR3 selected from the groups consisting of:

(1) QQ<3><1><1><1>P<1>T,

wherein <1> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY; <3> is 0.2 Y and 0.044 each of ADEFGHIKLMNPQRTVW;

(2) QQ33111P, wherein 1 and 3 are as defined in (1) above;

(3) QQ3211PP1T, wherein 1 and 3 are as defined in (1) above and 2 is 0.2 S and 0.044 each of ADEFGHIKLMNPQRTVWY; and

(4) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio CDR3s (1):(2):(3)::0.65:0.1:0.25.

7. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by variegated DNA sequences that encode a lambda light chain CDR1 selected from the group consisting of:

(1) TG<1>SS<2>VG<1><3><2><3>VS,

wherein <1> is 0.27 T, 0.27 G and 0.027 each of ADEFHIKLMNPQRSVWY, <2> is 0.27 D, 0.27 N and 0.027 each of AEFGHIKLMPQRSTVWY, and <3> is 0.36 Y and 0.036 each of ADEFGHIKLMNPQRSTVW;

(2) G<2><4>L<4><4><4><3><4><4>,

wherein <2> is as defined in (1) above and <4> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY; and

(3) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio CDR1s (1):(2)::0.67:0.33.

8. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by variegated DNA sequences that encode a lambda light chain CDR2 has the sequence:

<4><4><4><2>RPS,

wherein <2> is 0.27 D, 0.27 N, and 0.027 each of AEFGHIKLMPQRSTVWY and <4> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVW.

9. A focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, the vectors or genetic packages being characterized by variegated DNA sequences that encode a lambda light chain CDR3 selected from the group consisting of:

(1) <4><5><4><2><4>S<4><4><4><4>V,

wherein <2> is 0.27 D, 0.27 N, and 0.027 each of AEFGHIKLMPQRSTVWY; <4> is an equimolar mixture of amino

acid residues ADEFGHIKLMNPQRSTVW; and <5> is 0.36 S and 0.0355 each of ADEFGHIKLMNPQRTVWY;

(2) <5>SY<1><5>S<5><1><4>V, wherein <1> is an equimolar mixture of ADEFGHIKLMNPQRSTVWY; and <4> and <5> are as defined in (1) above; and

(3) mixtures of vectors or genetic packages characterized by any of the above DNA sequences, preferably in the ratio CDR3s (1):(2)::1:1.

10. A focused library comprising variegated DNA sequences that encode a heavy chain CDR selected from the group consisting of:

(1) one or more of the heavy chain CDR1s of paragraph 1 above;

(2) one or more of the heavy chain CDR2s of paragraph 2 above;

(3) one or more of the heavy chain CDR3s of paragraph 3 above; and

(4) mixtures of vectors or genetic packages characterized by (1), (2) and (3).

11. The focused library comprising one or more of the variegated DNA sequences that encodes a heavy chain CDR of paragraphs 1, 2 and 3 and further comprising variegated DNA sequences that encodes a light chain CDR selected from the group consisting of

(1) one or more the kappa light chain CDR1s of paragraph 4;

(2) the kappa light chain CDR2 of paragraph 5;

(3) one or more of the kappa light chain CDR3s of paragraph 6;

(4) one or more of the kappa light chain CDR1s of paragraph 7;

(5) the lambda light chain CDR2 of paragraph 8;

(6) one or more of the lambda light chain CDR3s of paragraph 9; and

(7) mixtures of vectors and genetic packages characterized by one or more of (1) through (6).

12. A population of variegated DNA sequences as described in paragraphs 1-11 above.

13. A population of vectors comprising the variegated DNA sequences as described in paragraphs 1-11 above.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Antibodies ("Ab") concentrate their diversity into those regions that are involved in determining affinity and specificity of the Ab for particular targets. These regions may be diverse in sequence or in length. Generally, they are diverse in both ways. However, within families of human antibodies the diversities, both in sequence and in length, are not truly random. Rather, some amino acid residues are preferred at certain positions of the CDRs and some CDR lengths are preferred. These preferred diversities account for the natural diversity of the antibody family.

According to this invention, and as more fully described below, libraries of vectors and genetic packages that more closely mirror the natural diversity, both in

sequence and in length, of antibody families, or portions thereof are prepared and used.

Human Antibody Heavy Chain Sequence and Length Diversity

(a) Framework

The heavy chain ("HC") Germ-Line Gene (GLG) 3-23 (also known as VP-47) accounts for about 12% of all human Abs and is preferred as the framework in the preferred embodiment of the invention. It should, however, be understood that other well-known frameworks, such as 4-34, 3-30, 3-30.3 and 4-30.1, may also be used without departing from the principles of the focused diversities of this invention.

In addition, JH4 (YFDYWGQGTLVTUSS) occurs more often than JH3 in native antibodies. Hence, it is preferred for the focused libraries of this invention. However, JH3 (AFDIWGQGTMVTVSS) could as well be used.

(b) Focused Length Diversity: CDR1, 2 and 3

(i) CDR1

For CDR1, GLGs provide CDR1s only of the lengths 5, 6, and 7. Mutations during the maturation of the V-domain gene, however, can lead to CDR1s having lengths as short as 2 and as long as 16. Nevertheless, length 5 predominates. Accordingly, in the preferred embodiment of this invention, the preferred HC CDR1 is 5 amino acids, with less preferred CDR1s having lengths of 7 and 14. In the most preferred libraries of this invention, all three lengths are used in proportions similar to those found in natural antibodies.

(ii) CDR2

GLGs provide CDR2s only of the lengths 15-19, but mutations during maturation may result in CDR2s of lengths from 16 to 28 amino acids. The lengths 16 and 17 predominate in mature Ab genes. Accordingly, length 17 is the preferred length for HC CDR2 of the present invention. Less preferred HC CDR2s of this invention have lengths 16 and 19. In the most preferred focused libraries of this invention, all three lengths are included in proportions similar to those found in natural antibody families.

(iii) CDR3

HC CDR3s vary in length. About half of human HCs consist of the components: V::nz::D::ny::JHn where V is a V gene, nz is a series of bases (mean 12) that are essentially random, D is a D segment, often with heavy editing at both ends, ny is a series of bases (mean 6) that are essentially random, and JH is one of the six JH segments, often with heavy editing at the 5' end. The D segments appear to provide spacer segments that allow folding of the IgG. The greatest diversity is at the junctions of V with D and of D with JH.

In the preferred libraries of this invention both types of HC CDR3s are used. In HC CDR3s that have no identifiable D segment, the structure is V::nz::JHn where JH is usually edited at the 5' end. In HC CDR3s that have an identifiable D segment, the structure is V::nz::D::ny::JHn.

(c) Focused Sequence Diversity: CDR1, 2 and 3

(i) CDR1

In 5 amino acid length CDR1, examination of a 3D model of a humanized Ab showed that the side groups of residues 1, 3, and 5 were directed toward the combining pocket. Consequently, in the focused libraries of this invention, each of these positions may be selected from any of the native amino acid residues, except cysteine ("C"). Cysteine can form disulfide bonds, which are an important component of the canonical Ig fold. Having free thiol groups could, thus, interfere with proper folding of the HC and could lead to problems in production or manipulation of selected Abs. Thus, in the focused libraries of this invention cysteine is excluded from positions 1, 3 and 5 of the preferred 5 amino acid CDR1s. The other 19 natural amino acids residues may be used at positions 1, 3 and 5. Preferably, each is present in equimolar ratios in the variegated libraries of this invention.

3D modeling also suggests that the side groups of residue 2 in a 5 amino acid CDR1 are directed away from the combining pocket. Although this position shows substantial diversity, both in GLG and mature genes, in the focused libraries of this invention this residue is preferably Tyr (Y) because it occurs in 681/820 mature antibody genes. However, any of the other native amino acid residues, except Cys (C), could also be used at this position.

For position 4, there is also some diversity in GLG and mature antibody genes. However, almost all mature genes have uncharged hydrophobic amino acid residues: A, G, L, P, F, M, W, I, V, at this position. Inspection of a 3D model also shows that the side group of residue 4 is packed into the innards of the HC. Thus, in the preferred embodiment of this invention which uses framework 3-23,

residue 4 is preferably Met because it is likely to fit very well into the framework of 3-23. With other frameworks, a similar fit consideration is used to assign residue 4.

Thus, the most preferred HC CDR1 of this invention consists of the amino acid sequence <1>Y<1>M<1> where <1> can be any one of amino acid residues: A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y (not C), preferably present at each position in an equimolar amount. This diversity is shown in the context of a framework 3-23:JH4 in Table 1. It has a diversity of 6859-fold.

The two less preferred HC CDR1s of this invention have length 7 and length 14. For length 7, a preferred variegation is $(S/T)_1(S/G/<1>)_2(S/G/<1>)_3Y_4Y_5W_6(S/G/<1>)_7$; where (S/T) indicates an equimolar mixture of Ser and Thr codons; (S/G/<1>) indicates a mixture of 0.2025 S, 0.2025 G, and 0.035 for each of A, D, E, F, H, I, K, L, M, N, P, Q, R, T, V, W, Y. This design gives a predominance of Ser and Gly at positions 2, 3, and 7, as occurs in mature HC genes. For length 14, a preferred variegation is VSGGSIS<1><1><1>YYW<1>, where <1> is an equimolar mixture of the 19 native amino acid residues, except Cys (C).

The DNA that encodes these preferred HC CDR1s is preferably synthesized using trinucleotide building blocks so that each amino acid residue is present in essentially equimolar or other described amounts. The preferred codons for the <1> amino acid residues are gct, gat, gag, ttt, ggt, cat, att, aag, ctt, atg, aat, cct, cag, cgt, tct, act, gtt, tgg, and tat. Of course, other codons for the chosen amino acid residue could also be used.

The diversity oligonucleotide (ON) is preferably synthesized *from BspEI to BstXI* (as shown in Table 1) and can, therefore, be incorporated either by PCR synthesis

using overlapping ONs or introduced by ligation of *Bsp*EI/*Bst*XI-cut fragments. Table 2 shows the oligonucleotides that embody the specified variegations of the preferred length 5 HC CDR1s of this invention. PCR using ON-R1V1vg, ON-R1top, and ON-R1bot gives a dsDNA product of 73 base pairs, cleavage with *Bsp*EI and *Bst*XI trims 11 and 13 bases from the ends and provides cohesive ends that can be ligated to similarly cut vector having the 3-23 domain shown in Table 1. Replacement of ON-R1V1vg with either ONR1V2vg or ONR1V3vg (see Table 2) allows synthesis of the two alternative diversity patterns -- the 7 residue length and the 14 residue length HC CDR1.

The more preferred libraries of this invention comprise the 3 preferred HC CDR1 length diversities. Most preferably, the 3 lengths should be incorporated in approximately the ratios in which they are observed in antibodies selected without reference to the length of the CDRs. For example, one sample of 1095 HC genes have the three lengths present in the ratio: L=5:L=7:L=14::820:175:23::0.80:0.17:0.02. This is the preferred ratio in accordance with this invention.

(ii) CDR2

Diversity in HC CDR2 was designed with the same considerations as for HC CDR1: GLG sequences, mature sequences and 3D structure. A preferred length for CDR2 is 17, as shown in Table 1. For this preferred 17 length CDR2, the preferred variegation in accordance with the invention is: <2>I<2><3>SGG<1>T<1>YADSVKG, where <2> indicates any amino acid residue selected from the group of Y, R, W, V, G and S (equimolar mixture), <3> is P, S and G or P and S only (equimolar mixture), and <1> is any native amino acid residue except C (equimolar mixture).

ON-R2V1vg shown in Table 3 embodies this diversity pattern. It is preferably synthesized so that fragments of dsDNA containing the *Bst*XI and *Xba*I site can be generated by PCR. PCR with ON-R2V1vg, ON-R2top,and ONR2bot gives a dsDNA product of 122 base pairs. Cleavage with *Bst*XI and *Xba*I removes about 10 bases from each end and produces cohesive ends that can be ligated to similarly cut vector that contains the 3-23 gene shown in Table 1.

In an alternative embodiment for a 17 length HC CDR2, the following variegation may be used: <1>I<4><1><1>G<5><1><1><1>YADSVKG, where <1> is as described above for the more preferred alternative of HC CDR2; <4> indicates an equimolar mixture of DINSWY, and <5> indicates an equimolar mixture of SGDN. This diversity pattern is embodied in ON-R2V2vg shown in Table 3. Preferably, the two embodiments are used in equimolar mixtures in the libraries of this invention.

Other preferred HC CDR2s have lengths 16 and 19. Length 16: <1>I<4><1><1>G<5<1><1>YNPSLKG; Length 19: <1>I<8>S<1><1><1>GGYY<1>YAASVKG, wherein <1> is an equimolar mixture of all native amino acid residues except C; <4> is a equimolar mixture of DINSWY; <5> is an equimolar mixture of SGDN; and <8> is 0.27 R and 0.027 of each of residues ADEFGHIKLMNPQSTVWY. Table 3 shows ON-R2V3vg which embodies a preferred CDR2 variegation of length 16 and ON-R2V4vg which embodies a preferred CDR2 variegation of length 19. To prepare these variegations ON-R2V3vg may be PCR amplified with ON-R2top and ON-R2bo3 and ON-R2V4vg may be PCR amplified with ON-R2top and ON-R2-bo4. See Table 3. In the most preferred embodiment of this invention, all three HC CDR2 lengths are used. Preferably, they are present in a ratio 17:16:19::579:464:31::0.54:0.43:0.03.

(iii) CDR3

The preferred libraries of this invention comprise several HC CDR3 components. Some of these will have only sequence diversity. Others will have sequence diversity with embedded D segments to extend the length, while also incorporating sequences known to allow Igs to fold. The HC CDR3 components of the preferred libraries of this invention and their diversities are depicted in Table 4: Components 1-8.

This set of components was chosen after studying the sequences of 1383 human HC sequences. The proposed components are meant to fulfill the following goals:

1) approximately the same distribution of lengths as seen in native Ab genes;

2) high level of sequence diversity at places having high diversity in native Ab genes; and

3) incorporation of constant sequences often seen in native Ab genes.

Component 1 represents all the genes having lengths 0 to 8 (counting from the YYCAR motif at the end of FR3 to the WG dipeptide motif near the start of the J region, i.e., FR4). Component 2 corresponds the all the genes having lengths 9 or 10. Component 3 corresponds to the genes having lengths 11 or 12 plus half the genes having length 13. Component 4 corresponds to those having length 14 plus half those having length 13. Component 5 corresponds to the genes having length 15 and half of those having length 16. Component 6 corresponds to genes of length 17 plus half of those with length 16. Component 7 corresponds to those with length 18. Component 8 corresponds to those having length 19 and greater. See Table 4.

For each HC CDR3 residue having the diversity <1>, equimolar ratios are preferably not used. Rather, the following ratios are used 0.095 [G and Y] and 0.048 [A, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, and W]. Thus, there is a double dose of G and Y with the other residues being in equimolar ratios. For the other diversities, e.g., KR or SG, the residues are present in equimolar mixtures.

In the preferred libraries of this invention the eight components are present in the following fractions: 1 (0.10), 2 (0.14), 3 (0.25), 4 (0.13), 5 (0.13), 6 (0.11), 7 (0.04) and 8 (0.10). See Table 4.

In the more preferred embodiment of this invention, the amounts of the eight components is adjusted because the first component is not complex enough to justify including it as 10% of the library. For example, if the final library were to have 1×10^9 members, then 1×10^8 sequences would come from component 1, but it has only 2.6×10^5 CDR3 sequences so that each one would occur in ~385 CDR1/2 contexts. Therefore, the more preferred amounts of the eight components are 1(0.02), 2(0.14), 3(0.25), 4(0.14), 5(0.14), 6(0.12), 7(0.08), 8(0.11). In accordance with the more preferred embodiment component 1 occurs in ~77 CDR1/2 contexts and the other, longer CDR3s occur more often.

Table 5 shows vgDNA that embodies each of the eight HC CDR3 components shown in Table 4. In Table 5, the oligonucleotides (ON) Ctop25, CtprmA, CBprmB, and CBot25 allow PCR amplification of each of the variegated ONs (vgDNA): C1t08, C2t10, C3t12, C4t14, C5t15, C6t17, C7t18, and C8t19. After amplification, the dsDNA can be cleaved with *Afl*II and *Bst*EII (or *Kpn*I) and ligated to similarly cleaved vector that contains the remainder of the 3-23

domain. Preferably, this vector already contains diversity in one, or both, of CDR1 and CDR2 as disclosed herein. Most preferably, it contains diversity in both the CDR1 and CDR2 regions. It is, of course, to be understood that the various diversities can be incorporated into the vector in any order.

Preferably, the recipient vector originally contains a stuffer in place of CDR1, CDR2 and CDR3 so that there will be no parental sequence that would then occur in the resulting library. Table 6 shows a version of the V3-23 gene segment with each CDR replaced by a short segment that contains both stop codons and restriction sites that will allow specific cleavage of any vector that does not have the stuffer removed. The stuffer can either be short and contain a restriction enzyme site that will not occur in the finished library, allowing removal of vectors that are not cleaved by both *Afl*II and *Bst*EII (or *Kpn*I) and religated. Alternatively, the stuffer could be 200-400 bases long so that uncleaved or once cleaved vector can be readily separated from doubly cleaved vector.

Human Antibody Light Chain: Sequence and Length Diversity

(i) Kappa Chain

(a) Framework

In the preferred embodiment of this invention, the kappa light chain is built in an A27 framework with a JK1 region. These are the most common V and J regions in the native genes. Other frameworks, such as O12, L2, and A11, and other J regions, such as JK4, however, may be used without departing from the scope of this invention.

(b) CDR1

In native human kappa chains, CDR1s with lengths of 11, 12, 13, 16, and 17 were observed with length 11

being predominant and length 12 being well represented. Thus, in the preferred embodiments of this invention LC CDR1s of length 11 and 12 are used in an and mixture similar to that observed in native antibodies), length 11 being most preferred. Length 11 has the following sequence: RASQ<1>V<2><2><3>LA and Length 12 has the following sequence: RASQ<1>V<2><2><2><3>LA, wherein <1> is an equimolar mixture of all of the native amino acid residues, except C, <2> is 0.2 S and 0.044 of each of ADEFGHIKLMNPQRTVWY, and <3> is 0.2 Y and 0.044 each of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W and Y. In the most preferred embodiment of this invention, both CDR1 lengths are used. Preferably, they are present in a ratio of 11:12::154:73::0.68:0.32.

(c) CDR2

In native kappa, CDR2 exhibits only length 7. This length is used in the preferred embodiments of this invention. It has the sequence <1>AS<2>R<4><1>, wherein <1> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY; <2> is 0.2 S and 0.004 of each of ADEFGHIKLMNPQRTVWY; and <4> is 0.2 A and 0.044 of each of DEFGHIKLMNPQRSTUWY.

(d) CDR3

In native kappa, CDR3 exhibits lengths of 1, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 19. While any of these lengths and mixtures of them can be employed in this invention, we prefer lengths 8, 9 and 10, length 9 being more preferred. For the preferred Length 9, the sequence is QQ<3><1><1><1>P<1>T, wherein <1> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY and <3> is 0.2 Y and 0.044 each of ADEFGHIKLMNPQRSVW. Length 8 is preferably QQ33111P and Length 10 is preferably QQ3211PP1T, wherein 1 and 3 are as defined for Length 9 and 2 is S

(0.2) and 0.044 each of ADEFGHIKLMNPQRTVWY. A mixture of all 3 lengths being most preferred (ratios as in native antibodies), i.e., 8:9:10::28:166:63::0.1:0.65:0.25.

Table 7 shows a kappa chain gene of this invention, including a PlacZ promoter, a ribosome-binding site, and signal sequence (M13 III signal). The DNA sequence encodes the GLG amino acid sequence, but does not comprise the GLG DNA sequence. Restriction sites are designed to fall within each framework region so that diversity can be cloned into the CDRs. XmaI and *Esp*I are in FR1, *Sex*AI is in FR2, *Rsr*II is in FR3, and *Kpn*I (or Acc65I) are in FR4. Additional sites are provided in the constant kappa chain to facilitate construction of the gene.

Table 7 also shows a suitable scheme of variegation for kappa. In CDR1, the most preferred length 11 is depicted. However, most preferably both lengths 11 and 12 are used. Length 12 in CDR1 can be construed by introducing codon 51 as <2> (*i.e.* a Ser-biased mixture). CDR2 of kappa is always 7 codons. Table 7 shows a preferred variegation scheme for CDR2. Table 7 shows a variegation scheme for the most preferred CDR3 (length 9). Similar variegations can be used for CDRs of length 8 and 10. In the preferred embodiment of this invention, those three lengths (8, 9 and 10) are included in the libraries of this invention in the native ratios, as described above.

Table 9 shows series of diversity oligonucleotides and primers that may be used to construct the kappa chain diversities depicted in Table 7.

(ii) Lambda Chain

(a) Framework

The lambda chain is preferably built in a 2a2 framework with an L2J region. These are the most common V

and J regions in the native genes. Other frameworks, such as 3l, 4b, 1a and 6a, and other J regions, such as L1J, L3J and L7J, however, may be used without departing from the scope of this invention.

(b) CDR1

In native human lambda chains, CDR1s with length 14 predominate, lengths 11, 12 and 13 also occur. While any of these can be used in this invention, lengths 11 and 14 are preferred. For length 11 the sequence is: TG<2><4>L<4><4><4><3><4><4> and for Length 14 the sequence is: TG<1>SS<2>VG<1><3><2><3>VS, wherein <1> is 0.27 T, 0.27 G and 0.027 each of ADEFHIKLMNPQRSVWY; <2> is 0.27 D, 0.27 N and 0.027 each of AEFGHIKLMPQRSTVWY; <3> is 0.36 Y and 0.0355 each of ADEFGHIKLMNPQRSTVW; and <4> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVWY. Most preferably, mixtures (similar to those occurring in native antibodies) preferably, the ratio is 11:14::23:46::0.33:0.67 of the three lengths are used.

(c) CDR2

In native human lambda chains, CDR2s with length 7 are by far the most common. This length is preferred in this invention. The sequence of this Length 7 CDR2 is <4><4><4><2>RPS, wherein <2> is 0.27 D, 0.27 N, and 0.027 each of AEFGHIKLMPQRSTVWY and <4> is an equimolar mixture of amino acid residues ADEFGHIKLMNPQRSTVW.

(d) CDR3

In native human lambda chains, CDR3s of length 10 and 11 predominate, while length 9 is also common. Any of these three lengths can be used in the invention. Length 11 is preferred and mixtures of 10 and 11 more preferred. The sequence of Length 11 is <4><5><4><2><4>S<4><4><4><4>V, where <2> and <4> are as defined for the lambda CDR1 and <5> is 0.36 S and 0.0355 each of ADFFGHIKLMNPQRTVWY. The

sequence of Length 10 is <5>SY<1><5>S<5><1><4>V, wherein <1> is an equimolar mixture of ADEFGHIKLMNPQRSTVWY; and <4> and <5> are as defined for Length 11. The preferred mixtures of this invention comprise an equimolar mixture of Length 10 and Length 11. Table 8 shows a preferred focused lambda light chain diversity in accordance with this invention.

Table 9 shows a series of diversity oligonucleotides and primers that may be used to construct the lambda chain diversities depicted in Table 7.

Method of Construction of the Genetic Package

The diversities of heavy chain and the kappa and lambda light chains are best constructed in separate vectors. First a synthetic gene is designed to embody each of the synthetic variable domains. The light chains are bounded by restriction sites for *Apa*LI (positioned at the very end of the signal sequence) and *Asc*I (positioned afer the stop codon). The heavy chain is bounded by *Sfi*I (positioned within the PelB signal sequence) and *Not*I (positioned in the linker between CH1 and the anchor protein). Signal sequences other than Pe1B may also need, e.g., a M13 pIII signal sequence.

The initial genes are made with "stuffer" sequences in place of the desired CDRs. A "Stuffer" is a sequence that is to be cut away and replaced by diverse DNA but which does not allow expression of a functional antibody gene. For example, the stuffer may contain several stop codons and restriction sites that will not occur in the correct finished library vector. For example, in Table 10, the stuffer for CDR1 of kappa A27 contains a *Stu*I site. The vgDNA for CDR1 is introduced as a cassette from *Esp*I, *Xma*I, or *Afl*II to either *Sex*AI or *Kas*I. After

the ligation, the DNA is cleaved with *StuI*; there should be no *StuI* sites in the desired vectors.

The sequences of the heavy chain gene with stuffers is depicted in Table 6. The sequences of the kappa light chain gene with stuffers is depicted in Table 10. The sequence of the lambda light chain gene with stuffers is depicted in Table 11.

In another embodiment of the present intention the diversities of heavy chain and the kappa or lambda light chains are constructed in a single vector or genetic packages (e.g., for display or display and expression) having appropriate restriction sites that allow cloning of these chains. The processes to construct such vectors are well known and widely used in the art. Preferably, a heavy chain and Kappa light chain library and a heavy chain and lambda light chain library would be prepared separately. The two libraries, most preferably, will then be mixed in equimolar amounts to attain maximum diversity.

Most preferably, the display is had on the surface of a derivative of M13 phage. The most preferred vector contains all the genes of M13, an antibiotic resistance gene, and the display cassette. The preferred vector is provided with restriction sites that allow introduction and excision of members of the diverse family of genes, as cassettes. The preferred vector is stable against rearrangement under the growth conditions used to amplify phage.

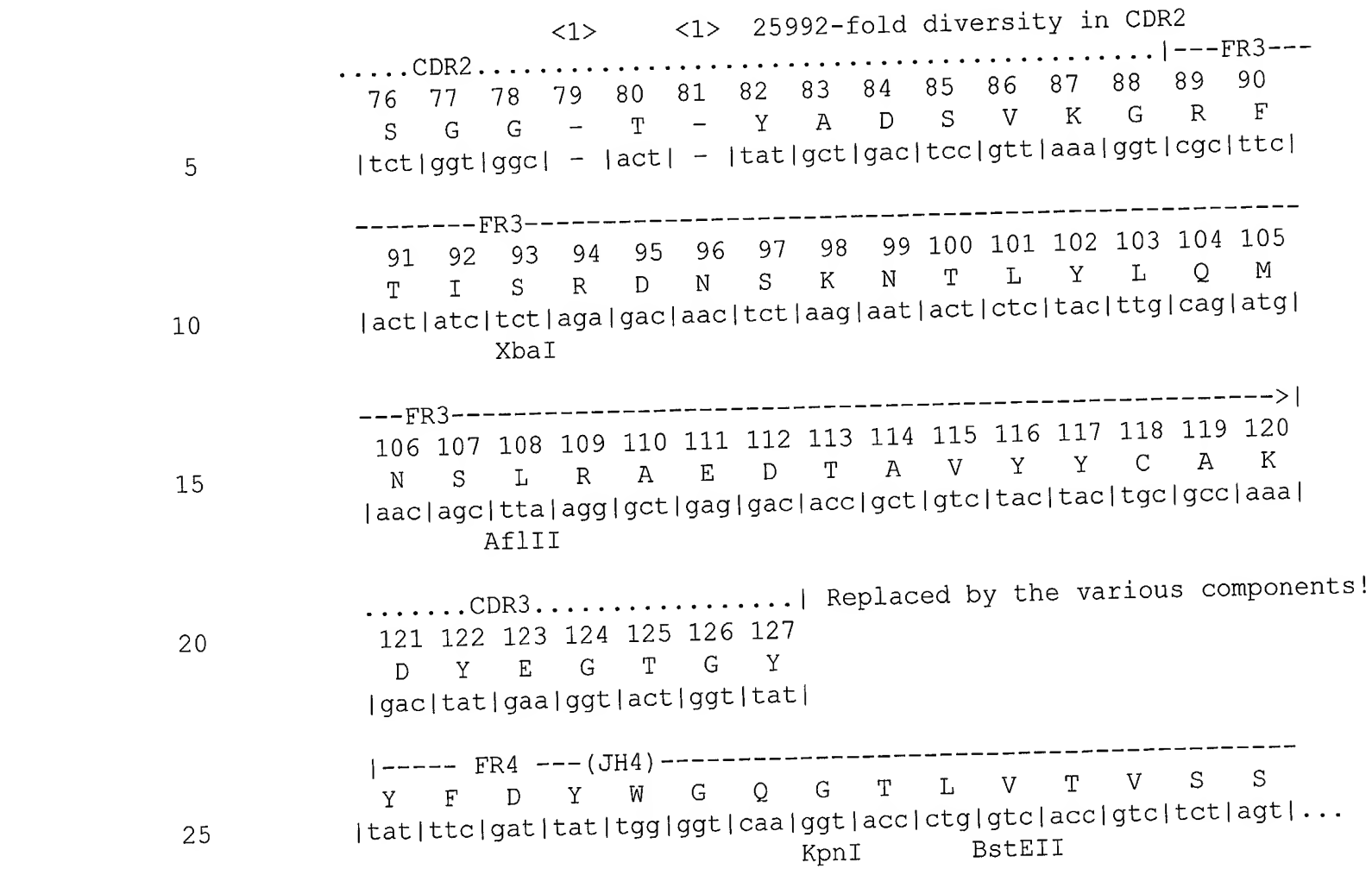
In another embodiment of this invention, the diversity captured by the methods of the present invention may be displayed and/or expressed in a phagemid vector (e.g., pCES1) that displays and/or expresses the peptide, polypeptide or protein. Such vectors may also be used to

store the diversity for subsequent display and/or expression using other vectors or phage.

In another embodiment of this invention, the diversity captured by the methods of the present invention may be displayed and/or expressed in a yeast vector.

Table 1: 3-23:JH4   CDR1/2 diversity = 1.78 x 10^8

```
                                     FR1(VP47/V3-23)---------------
                                     23  24  25  26  27  28  29  30
               20  21  22             E   V   Q   L   L   E   S   G
                A   M   A            gaa|gtt|caa|ttg|tta|gag|tct|ggt|
ctgtctgaac     cc atg gcc                     MfeI
Scab......     NcoI....

      -------------FR1-------------------------------------------------
       31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
        G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
      |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|

          Sites of variegation        <1>       <1> <1> <1>              6859-fold diversity
      ----FR1------------------->|.....CDR1....................|---FR2------
       46  47  48  49  50  51  52   53  54   55  56  57  58  59  60
        A   S   G   F   T   F   S   -   Y   -   M   -   W   V   R
      |gct|tcc|gga|ttc|act|ttc|tct| - |tac| - |atg| - |tgg|gtt|cgc|
            BspEI                      BsiWI                        BstXI.

                          Sites of variegation-><2>     <2> <3>
      -------FR2----------------------------------->|...CDR2........
       61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
        Q   A   P   G   K   G   L   E   W   V   S   -   I   -   -
      |caa|gct|cct|ggt|aaa|ggt|ttg|gag|tgg|gtt|tct| - |atc| - | - |
   ...BstXI
```


<1> <1> 25992-fold diversity in CDR2
.....CDR2.....|---FR3---
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
S G G - T - Y A D S V K G R F
|tct|ggt|ggc| - |act| - |tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|
--------FR3----------
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T I S R D N S K N T L Y L Q M
|act|atc|tct|aga|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
XbaI
---FR3---------->|
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
N S L R A E D T A V Y Y C A K
|aac|agc|tta|agg|gct|gag|gac|acc|gct|gtc|tac|tac|tgc|gcc|aaa|
AflII
.....CDR3.....| Replaced by the various components!
121 122 123 124 125 126 127
D Y E G T G Y
|gac|tat|gaa|ggt|act|ggt|tat|
|----- FR4 ---(JH4)----------
Y F D Y W G Q G T L V T V S S
|tat|ttc|gat|tat|tgg|ggt|caa|ggt|acc|ctg|gtc|acc|gtc|tct|agt|...
KpnI BstEII

<1> = Codons for ADEFGHIKLMNPQRSTVWY (equimolar mixture)
<2> = Codons for YRWVGS (equimolar mixture)
<3> = Codons for PS or PS and G (equimolar mixture)

Table 2: Oligonucleotides used to variegate CDR1 of human HC

CDR1 - 5 residues

(ON-R1V1vg): 5'-ct|tcc|gga|ttc|act|ttc|tct|<1>|tac|<1>|atg|<1>|tgg|gtt|cgc|caa|gct|cct|gg-3'

<1> = Codons of ADEFGHIKLMNPQRSTVWY 1:1

(ON-R1top): 5'-cctactgtct|tcc|gga|ttc|act|ttc|tct-3'

(ON-R1bot)[RC]: 5'-tgg|gtt|cgc|caa|gct|cct|ggttgctcactc-3'

CDR1 - 7 residues

(ON-R1V2vg): 5'-ct|tcc|gga|ttc|act|ttc|tct|<6>|<7>|<7>|tac|tac|tgg|<7>|tgg|gtt|cgc|caa|gct|cct|gg-3'

<6> = Codons for ST, 1:1

<7> = 0.2025(Codons for SG) + 0.035(Codons for ADEFHIKLMNPQRTVWY)

CDR1 - 14 residues

(ON-R1V3vg): 5'-ct|tcc|gga|ttc|act|ttc|tct|atc|agc|ggt|ggt|tct|atc|tcc|<1>|<1>|<1>|-tac|tac|tgg|<1>|tgg|gtt|cgc|caa|gct|cct|gg-3'

<1> = Codons for ADEFGHIKLMNPQRSTVWY 1:1

Table 3: Oligonucleotides used to variegate CDR2 of human HC

CDR2 - 17 residues

(ON-R2V1vg): 5'-ggt|ttg|gag|tgg|gtt|tct|<2>|atc|<2>|<3>|tct|ggt|ggc|<1>|act|<1>|tat|gct|-gac|tcc|gtt|aaa|gg-3'

(ON-R2top): 5'-ct|tgg|gtt|cgc|caa|gct|cct|ggt|aaa|ggt|ttg|gag|tgg|gtt|tct-3'

(ON-R2bot)[RC]: 5'-tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|act|atc|tct|aga|ttcctgtcac-3'

<1> = Codons for A,D,E,F,G,H,I,K,L,M,N,P,Q,R,S,T,V,W and Y (equimolar mixture)

<2> = Codons for Y,R,W,V,G and S (equimolar mixture)

<3> = Codons for P and S (equimolar mixture) or P,S and G (equimolar mixture)

(ON-R2V2vg): 5'-ggt|ttg|gag|tgg|gtt|tct|<1>|atc|<4>|<1>|<1>|ggt|<5>|<1>|<1>|<1>|tat|gct|-gac|tcc|gtt|aaa|gg-3'

<4> = Codons for DINSWY (equimolar mixture)

<5> = Codons for SGDN, (equimolar mixture)

CDR2 - 16 residues

(ON-R2V3vg): 5'-ggt|ttg|gag|tgg|gtt|tct|<1>|atc|<4>|<1>|<1>|ggt|
<5>|<1>|<1>|tat|aac|cct|tcc|ctt|aag|gg-3'

(ON-R2bo3)[RC]: 5'-tat|aac|cct|tcc|ctt|aag|ggt|cgc|ttc|act|atc|tct|aga|ttcctgtcac-3'

CDR2 - 19 residues

(ON-R2V4vg): 5'-ggt|ttg|gag|tgg|gtt|tct|<1>|atc|<8>|agt|<1>|<1>|
<1>|ggt|ggt|act|act|<1>|tat|gcc|gct|tcc|gtt|aag|gg-3'

(ON-R2bo4)[RC]: 5'-tat|gcc|gct|tcc|gtt|aag|ggt|cgc|ttc|act|atc|tct|aga|ttcctgtcac-3'

<1>, <2>, <3>, <4> and <5> are as defined above

<8> is 0.27 R and 0.027 each of ADEFGHIKLMNPQSTVWY

Table 4: Preferred Components of HC CDR3

Component		Length	Complexity	Fraction of Library	Preferred Adjusted Fraction
1	YYCA21111YFDYWG. (1=any amino acid residue, except C; 2 = K and R)	8	2.6×10^5	.10	.02
2	YYCA2111111YFDYWG. (1=any amino acid residue, except C; 2 = K and R)	10	9.4×10^7	.14	.14
3	YYCA211111111YFDYTG. (1=any amino acid residue, except C; 2 = K and R)	12	3.4×10^{10}	.25	.25
4	YYCAR111S2S3111YFDYWG. (1=any amino acid residue, except C; 2 = S and G 3 = Y and W)	14	1.9×10^8	.13	.14
5	YYCA2111CSG11CY1YFDYWG. (1=any amino acid residue, except C; 2 = K and R)	15	9.4×10^7	.13	.14
6	YYCA211S1TIFG11111YFDYWG. (1=any amino acid residue, except C; 2 = K and R)	17	1.7×10^{10}	.11	.12
7	YYCAR111YY2S33YY111YFDYWG. (1=any amino acid residue, except C; 2 = D or G; 3 = S and G)	18	3.8×10^8	.04	.08
8	YYCAR1111YC2231CY111YFDYWG. (1=any amino acid residue, except C; 2 = S and G; 3 = T, D and G)	19	2.0×10^{11}	.10	.11

Table 5: Oligonucleotides used to variegate the eight components of HC CDR3

(Ctop25): 5'-gctctggtcaac|tta|agg|gct|gag|g-3'

(CtprmA): 5'-gctctggtcaac|tta|agg|gct|gag|gac|acc|gct|gtc|tac|tac|tgc|gcc-3'
AflII...

(CBprmB)[RC]: 5'-|tac|ttc|gat|tac|tgg|ggc|caa|ggt|acc|ctg|gtc|acc|tcgctccacc-3'
BstEII...

(CBot25)[RC]: 5'-|ggt|acc|ctg|gtc|acc|tcgctccacc-3'

The 20 bases at 3' end of CtprmA are identical to the most 5' 20 bases of each of the vgDNA molecules.
Ctop25 is identical to the most 5' 25 bases of CtprmA.
The 23 most 3' bases of CBprmB are the reverse complement of the most 3' 23 bases of each of the vgDNA molecules.
CBot25 is identical to the 25 bases at the 5' end of CBprmB.

Component 1

(C1t08):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|<1>|<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of the residues ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 2

(C2t10):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|<1>|<1>|<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 3

(C3t12):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|<1>|<1>|<1>|<1>|<1>|<1>|tac|ttc|gat|tac|-tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 4

(C4t140):

5'-cc|gct|gtc|tac|tac|tgc|gcc|cgt|<1>|<1>|<1>|tct|<2>|tct|<3>|<1>|<1>|<1>|tac|ttc|gat|-tac|tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = S and G (equimolar mixture); <3> = Y and W (equimolar mixture)

Component 5

(C5t15):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|<1>|tgc|tct|ggt|<1>|<1>|tgc|tat|<1>|tac|-ttc|gat|tac|tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 6

(C6t17):

5'-cc|gct|gtc|tac|tac|tgc|gcc|<2>|<1>|<1>|tct|<1>|act|atc|ttc|ggt|<1>|<1>|<1>|<1>|-<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = K and R (equimolar mixture)

Component 7

(C7t18):

5'-cc|gct|gtc|tac|tac|tgc|gcc|cgt|<1>|<1>|<1>|tat|tac|<2>|tct|<3>|<3>|tac|tat|-<1>|<1>|<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = D and G (equimolar mixture); <3> = S and G (equimolar mixture)

Component 8
(c8t19):

5'-cc|gct|gtc|tac|tac|tgc|gcc|cgt|<1>|<1>|<1>|<1>|tat|tgc|<2>|<2>|<3>|<1>|tgc|tat|-<1>|<1>|<1>|tac|ttc|gat|tac|tgg|ggc|caa|gg-3'

<1> = 0.095 Y + 0.095 G + 0.048 each of ADEFHIKLMNPQRSTVW, no C; <2> = S and G (equimolar mixture); <3> = TDG (equimolar mixture);

Table 6: 3-23::JH4 Stuffers in place of CDRs

```
                                                FR1(DP47/V3-23)---------------
                                                23  24  25  26  27  28  29  30
                        20  21  22               E   V   Q   L   L   E   S   G
                         A   M   A
                ctgtctgaac  cc atg gcc  gaa|gtt|caa|ttg|tta|gag|tct|ggt|
                Scab......  NcoI....                    MfeI

            ----------FR1-----------------------------------------
             31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
              G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
            |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|

            ----FR1------------------>|...CDR1 stuffer....|---FR2------
             46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
              A   S   G   F   T   F   S   S   Y   A   |   |   W   V   R
            |gct|tcc|gga|ttc|act|ttc|tct|tcg|tac|gct|tag|taa|tgg|gtt|cgc|
                  BspEI                 BsiWI                       BstXI.
```

```
       -------FR2--------------------------------->|...CDR2 stuffer.
       61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
        Q   A   P   G   K   G   L   E   W   V   S   |   p   r   |
      |caa|gct|cct|ggt|aaa|ggt|ttg|gag|tgg|gtt|tct|taa|cct|agg|tag|
...BstXI                                                    AvrII..
.....CDR2 stuffer.....................................|---FR3---
       91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
        T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
      |act|atc|tct|aga|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
              XbaI

      ---FR3----------..> CDR3 Stuffer------------>|
       106 107 108 109 110
        N   S   L   R   A
      |aac|agc|tta|agg|gct|tag taa agg cct taa
            AflII               StuI...
```

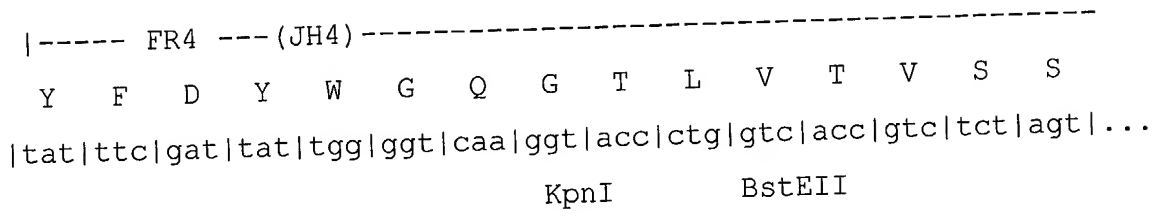

|----- FR4 ---(JH4)----------
Y F D Y W G Q G T L V T V S S
|tat|ttc|gat|tat|tgg|ggt|caa|ggt|acc|ctg|gtc|acc|gtc|tct|agt|...
KpnI
BstEII

Table 7: A27:JH1 Human Kappa light chain gene

gaggacc attgggcccc ctccgagact ctcgagcgca
Scab..... EcoO109I XhoI..
ApaI.

acgcaattaa tgtgagttag ctcactcatt aggcacccca ggctttacac tttatgcttc
..-35.. Plac ..-10.

cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacacagga

aacagctatg accatgatta

cgccaagctt tggagccttt tttttggaga ttttcaac
PflMI.....
Hind III

M13 III signal sequence (AA seq)---------->

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

M K K L L F A I P L V V P F Y

gtg aag aag ctc cta ttt gct atc ccg ctt gtc gtt ccg ttt tac

--Signal-->FR1---------->

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

S H S A Q S V L T Q S P G T L

|agc|cat|agt|gca|caa|tcc|gtc|ctt|act|caa|tct|cct|ggc|act|ctt|

ApaLI...

----- FR1 ---------->| CDR1------>

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

S L S P G E R A T L S C R A S

|tcg|cta|agc|ccg|ggt|gaa|cgt|gct|acc|tta|agt|tgc|cgt|gct|tcc|

EspI..... AflII...

XmaI....

For CDR1:

<1> ADEFGHIKLMNPQRSTVWY 1:1

<2> S(0.2) ADEFGHIKLMNPQRTVWY (0.044 each)

<3> Y(0.2) ADEFGHIKLMNPQRSTVW (0.044 each)

(CDR1 installed as AflII-(SexAI or KasI) cassette.) For the most preferred 11 length codon 51 (XXX) is omitted; for the preferred 12 length this codon is <2>

```
------- CDR1 ---------------------->|--- FR2 ---------------->
    <1>     <2> <2> xxx <3>
46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
 Q   -   V   -   -   -   -   L   A   W   Y   Q   Q   K   P
|cag| - |gtt| - | - | - | - |ctt|gct|tgg|tat|caa|cag|aaa|cct|
                                                    SexAI...
```

For CDR2:

<1> ADEFGHIKLMNPQRSTVWY 1:1

<2> S(0.2) ADEFGHIKLMNPQRTVWY (0.044 each)

<4> A(0.2) DEFGHIKLMNPQRSTVWY (0.044 each)

CDR2 installed as (SexAI or KasI) to (BamHI or RsrII) cassette.)

```
      ----- FR2 ----------------------->|------- CDR2 ---------->
                                        <1>       <2>     <4>
        61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
         G   Q   A   P   R   L   L   I   Y   -   A   S   -   R   -
       |ggt|cag|gcg|ccg|cgt|tta|ctt|att|tat| - |gct|tct| - |cgc| - |
    SexAI....   KasI....
```

```
CDR2-->|--- FR3 ---------------------------------------->
    <1>
    76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
     -   G   I   P   D   R   F   S   G   S   G   S   G   T   D
    | - |ggg|atc|ccg|gac|cgt|ttc|tct|ggc|tct|ggt|tca|ggt|act|gac|
          BamHI...
                RsrII.....


      ------ FR3 ---------------------------------------->
       91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
        F   T   L   T   I   S   R   L   E   P   E   D   F   A   V
      |ttt|acc|ctt|act|att|tct|aga|ttg|gaa|cct|gaa|gac|ttc|gct|gtt|
                                XbaI...
```

For CDR3 (Length 9):

<1> ADEFGHIKLMNPQRSTVWY 1:1

<3> Y(0.2) ADEFGHIKLMNPQRTVW (0.044 each)

For CDR3 (Length 8): QQ33111P

1 and 3 as defined for Length 9

For CDR3 (Length 10): QQ3211PP1T

1 and 3 as defined for Length 9

2 S(0.2) and 0.044 each of ADEFGHIKLMNPQRTVWY

CDR3 installed as XbaI to (StyI or BsiWI) cassette.

```
----------->|----CDR3------------------------->|-----FR4--->
                          <3> <1> <1> <1>     <1>
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
  Y   Y   C   Q   Q   -   -   -   -   P   -   T   F   G   Q
|tat|tat|tgc|caa|cag| - | - | - | - |cct| - |act|ttc|ggt|caa|
          BstXI.....
```

```
-----FR4------------------>|        <------- Ckappa ------------
121 122 123 124 125 126 127          128 129 130 131 132 133 134
  G   T   K   V   E   I   K            R   T   V   A   A   P   S
|ggt|acc|aag|gtt|gaa|atc|aag|      |cgt|acg|gtt|gcc|gct|cct|agt|
      StyI....                       BsiWI..

135 136 137 138 139 140 141 142 143 144 145 146 147 148 149
  V   F   I   F   P   P   S   D   E   Q   L   K   S   G   T
|gtg|ttt|atc|ttt|cct|cct|tct|gac|gaa|caa|ttg|aag|tca|ggt|act|
                                    MfeI...

150 151 152 153 154 155 156 157 158 159 160 161 162 163 164
  A   S   V   V   C   L   L   N   N   F   Y   P   R   E   A
|gct|tct|gtc|gta|tgt|ttg|ctc|aac|aat|ttc|tac|cct|cgt|gaa|gct|
                                            BssSI...
```

165 166 167 168 169 170 171 172 173 174 175 176 177 178 179

K V Q W K V D N A L Q S G N S

|aaa|gtt|cag|tgg|aaa|gtc|gat|aac|gcg|ttg|cag|tcg|ggt|aac|agt|

MluI....

180 181 182 183 184 185 186 187 188 189 190 191 192 193 194

Q E S V T E Q D S K D S T Y S

|caa|gaa|tcc|gtc|act|gaa|cag|gat|agt|aag|gac|tct|acc|tac|tct|

195 196 197 198 199 200 201 202 203 204 205 206 207 208 209

L S S T L T L S K A D Y E K H

|ttg|tcc|tct|act|ctt|act|tta|tca|aag|gct|gat|tat|gag|aag|cat|

210 211 212 213 214 215 216 217 218 219 220 221 222 223 224

K V Y A C E V T H Q G L S S P

|aag|gtc|tat|GCt|TGC|gaa|gtt|acc|cac|cag|ggt|ctg|agc|tcc|cct|

SacI....

225 226 227 228 229 230 231 232 233 234

V T K S F N R G E C . .

|gtt|acc|aaa|agt|ttc|aac|cgt|ggt|gaa|tgc|taa|tag ggcgcgcc

DsaI.... AscI....

BssHII

5

acgcatctctaa gcggccgc aacaggaggag

NotI....

Table 8: 2a2:JH2 Human lambda-chain gene

```
gaggaccatt gggcccc   ttactccgtgac
Scab..... EcoO109I
          ApaI..

          ----------FR1---------------------------------------->
                1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
           S   A   Q   S   A   L   T   Q   P   A   S   V   S   G   S   P   G
          agt|gca|caa|tcc|gct|ctc|act|cag|cct|gct|agc|gtt|tcc|ggg|tca|cct|ggt|
           ApaLI...                          NheI...          BstEII...
                                                                SexAI....
```

For CDR1 (length 14):

<1> = 0.27 T, 0.27 G, 0.027 each of ADEFHIKLMNPQRSVWY, no C

<2> = 0.27 D, 0.27 N, 0.027 each of AEFGHIKLMPQRSTVWY, no C

<3> = 0.36 Y, 0.0355 each of ADEFGHIKLMNPQRSTVW, no C

```
                             T   G  <1>  S   S  <2>  V   G
------FR1-------------------> |-----CDR1-----------------------
 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
  Q   S   I   T   I   S   C   T   G   -   S   S   -   V   G
|caa|agt|atc|act|att|tct|tgt|aca|ggt| - |tct|tct| - |gtt|ggc|
                      BsrGI..

<1> <3> <2> <3>  V   S = vg Scheme #1, length = 14
-----CDR1-------------->|--------FR2--------------------------
 31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
  -   -   -   -   V   S   W   Y   Q   Q   H   P   G   K   A
| - | - | - | - |gtt|tct|tgg|tat|caa|caa|cac|ccg|ggc|aag|gcg|
                                            XmaI....   KasI.....
                                            AvaI....
```

A second Vg scheme for CDR1 gives segments of length 11:

T_{22}G<2><4>L<4><4><4><3><4><4> where

<4> = equimolar mixture of each of ADEFGHIKLMNPQRSTVWY, no C

<3> = as defined above for the alternative CDR1

For CDR2:

<2> and <4> are the same variegation as for CDR1

```
                                  <4> <4> <4> <2>  R   P   S
          --FR2----------------> |------CDR2--------------->|-----FR3-
 5        46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
           P   K   L   M   I   Y   -   -   -   -   R   P   S   G   V
         |ccg|aag|ttg|atg|atc|tac| - | - | - | - |cgt|cct|tct|ggt|gtt|
     KasI....

         -------FR3-----------------------------------------------------
10       61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
          S   N   R   F   S   G   S   K   S   G   N   T   A   S   L
         |agc|aat|cgt|ttc|tcc|gga|tct|aaa|tcc|ggt|aat|acc|gca|agc|tta|
                        BspEI..                             HindIII.
                             BsaBI........(blunt)
```

-------FR3----------->|

76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
T	I	S	G	L	Q	A	E	D	E	A	D	Y	Y	C

|act|atc|tct|ggt|ctg|cag|gct|gaa|gac|gag|gct|gac|tac|tat|tgt|

PstI...

CDR3 (Length 11):

<2> and <4> are the same variegation as for CDR1

<5> = 0.36 S, 0.0355 each of ADEFGHIKLMNPQRTVWY no C

CDR3 (Length 10): <5> SY <1> <5> S <5> <1> <4> V

<1> is an equimolar mixture of ADEFGHIKLMNPQRSTVWY, no C

<4> and <5> are as defined for Length 11

<4> <5> <4> <2> <4> S <4> <4> <4> <4> V

```
-----CDR3---------------------------------->|---FR4---------
 91  92  93  94  95  96  97  98  99  100 101 102 103 104 105
  -   -   -   -   -   S   -   -   -   -   V   F   G   G   G
 | - | - | - | - | - |tct| - | - | - | - |gtc|ttc|ggc|ggt|ggt|
                                                      KpnI...

-------FR4-------------->
 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
  T   K   L   T   V   L   G   Q   P   K   A   A   P   S   V
 |acc|aaa|ctt|act|gtc|ctc|ggt|caa|cct|aag|gct|gct|cct|tcc|gtt|
KpnI...                  HincII..
                                Bsu36I...

 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
  T   L   F   P   P   S   S   E   E   L   Q   A   N   K   A
 |act|ctc|ttc|cct|cct|agt|tct|gaa|gag|ctt|caa|gct|aac|aag|gct|
                                SapI.....
```

```
136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
 T   L   V   C   L   I   S   D   F   Y   P   G   A   V   T
|act|ctt|gtt|tgc|ttg|atc|agt|gac|ttt|tat|cct|ggt|gct|gtt|act|
                  BclI....

151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
 V   A   W   K   A   D   S   S   P   V   K   A   G   V   E
|gtc|gct|tgg|aaa|gcc|gat|tct|tct|cct|gtt|aaa|gct|ggt|gtt|gag|
                                                  BsmBI...

166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
 T   T   T   P   S   K   Q   S   N   N   K   Y   A   A   S
|acg|acc|act|cct|tct|aaa|caa|tct|aac|aat|aag|tac|gct|gcg|agc|
                                              SacI....
BsmBI....

181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
 S   Y   L   S   L   T   P   E   Q   W   K   S   H   K   S
|tct|tat|ctt|tct|ctc|acc|cct|gaa|caa|tgg|aag|tct|cat|aaa|tcc|
SacI...
```

```
196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
 Y   S   C   Q   V   T   H   E   G   S   T   V   E   K   T
|tat|tcc|tgt|caa|gtt|act|cat|gaa|ggt|tct|acc|gtt|gaa|aag|act|
                        BspHI...
211 212 213 214 215 216 217 218 219
 V   A   P   T   E   C   S   .   .
|gtt|gcc|cct|act|gag|tgt|tct|tag|tga|ggcgcgcc
                                    AscI....
                                     BssHII

 aacgatgttc aag gcggccgc aacaggaggag
             NotI.... Scab.......
```

Table 9: Oligonucleotides For Kappa and Lambda Light Chain Variegation

(Ctop25): 5'-gctctggtcaac|tta|agg|gct|gag|g-3'

(CtprmA): 5'-gctctggtcaac|tta|agg|gct|gag|gac|acc|gct|gtc|tac|tac|tgc|gcc-3'

AflII...

(CBprmB)[RC]: 5'-|tac|ttc|gat|tac|ttg|ggc|caa|ggt|acc|ctg|gtc|acc|tcgctccacc-3'

BstEII...

(CBot25)[RC]: 5'-|ggt|acc|ctg|gtc|acc|tcgctccacc-3'

Kappa chains: CDR1 ("1"), CDR2 ("2"), CDR3 ("3")

CDR1

(Ka1Top610): 5'-ggtctcagttg|cta|agc|ccg|ggt|gaa|cgt|gct|acc|tta|agt|tgc|cgt|gct|tcc|cag-3'

(Ka1STp615): 5'-ggtctcagttg|cta|agc|ccg|ggt|g-3'

(Ka1Bot620) [RC]: 5'-ctt|gct|tgg|tat|caa|cag|aaa|cct|ggt|cag|gcg|ccaagtcgtgtc-3'

(Ka1SB625) [RC]: 5'-cct|ggt|cag|gcg|ccaagtcgtgtc-3'

(Ka1vg600): 5'-gct|acc|tta|agt|tgc|cgt|gct|tcc|cag-
|<1>|gtt|<2>|<2>|<3>|ctt|gct|tgg|tat|caa|cag|aaa|cc-3'
(Ka1vg600-12): 5'-gct|acc|tta|agt|tgc|cgt|gct|tcc|cag-
|<1>|gtt|<2>|<2>|<2>|<3>|ctt|gct|tgg|tat|caa|cag|aaa|cc-3'

CDR2

(Ka2Tshort657): 5'-cacgagtccta|cct|ggt|cag|gc-3'
(Ka2Tlong655): 5'-cacgagtccta|cct|ggt|cag|gcg|ccg|cgt|tta|ctt|att|tat-3'
(Ka2Bshort660):[RC]: 5'-|gac|cgt|ttc|tct|ggt|tctcacc-3'
(Ka2vg650): 5'-cag|gcg|ccg|cgt|tta|ctt|att|tat|<1>|gct|tct|<2>|-
|cgc|<4>|<1>|ggg|atc|ccg|gac|cgt|ttc|tct|ggt|tctcacc-3'

CDR3

(Ka3Tlon672): 5'-gacgagtccttct|aga|ttg|gaa|cct|gaa|gac|ttc|gct|gtt|tat|tat|tgc|caa|c-3'
(Ka3BotL682) [RC]: 5'-act|ttc|ggt|caa|ggt|acc|aag|gtt|gaa|atc|aag|cgt|acg|tcacaggtgag-3'
(Ka3Bsho694) [RC]: 5'-gaa|atc|aag|cgt|acg|tcacaggtgag-3'

(Ka3vg670): 5'-gac|ttc|gct|gtt|-
|tat|tat|tgc|caa|cag|<3>|<1>|<1>|<1>|cct|<1>|act|ttc|ggt|caa|-
|ggt|acc|aag|gtt|g-3'

(Ka3vg670-8): 5'-gac|ttc|gct|gtt|-
|tat|tat|tgc|caa|cag|<3>|<3>|<1>|<1>|<1>|cct|ttc|ggt|caa|-
|ggt|acc|aag|gtt|g-3'

(Ka3vg670-10): 5'-gac|ttc|gct|gtt|tat|-
|tat|tgc|caa|cag|<3>|<2>|<1>|<1>|cct|cct|<1>|act|ttc|ggt|caa|-
|ggt|acc|aag|gtt|g-3'

Lambda Chains: CDR1 ("1"), CDR2 ("2"), CDR3 ("3")

CDR1

(Lm1TPri75): 5'-gacgagtcctgg|tca|cct|ggt|-3'

(Lm1tlo715): 5'-gacgagtcctgg|tca|cct|ggt|caa|agt|atc|act|att|tct|tgt|aca|ggt-3'

(Lm1blo724) [rc]: 5'-gtt|tct|tgg|tat|caa|caa|cac|ccg|ggc|aag|gcg|agatcttcacaggtgag-3'

(Lm1bsh737) [rc]: 5'-gc|aag|gcg|agatcttcacaggtgag-3'

(Lm1vg710b):5'-gt|atc|act|att|tct|tgt|aca|ggt|<2>|<4>|ctc|<4>|<4>|<4>|-
|<3>|<4>|<4>|tgg|tat|caa|caa|cac|cc-3'

(Lm1vg710): 5'-gt|atc|act|att|tct|tgt|aca|ggt|<1>|tct|tct|<2>|gtt|ggc|-
|<1>|<3>|<2>|<3>|gtt|tct|tgg|tat|caa|caa|cac|cc-3'

CDR2

(Lm2TSh757): 5'-gagcagaggac|ccg|ggc|aag|gc-3'

(Lm2TLo753): 5'-gagcagaggac|ccg|ggc|aag|gcg|ccg|aag|ttg|atg|atc|tac|-3'

(Lm2BLo762) [RC]: 5'-cgt|cct|tct|ggt|gtc|agc|aat|cgt|ttc|tcc|gga|tcacaggtgag-3'

(Lm2BSh765) [RC]: 5'-cgt|ttc|tcc|gga|tcacaggtgag-3'

(Lm2vg750): 5'-g|ccg|aag|ttg|atg|atc|tac|-
<4>|<4>|<4>|<2>|cgt|cct|tct|ggt|gtc|agc|aat|c-3'

CDR3

(Lm3TSh822): 5'-ctg|cag|gct|gaa|gac|gag|gct|gac-3'

(Lm3TLo819): 5'-ctg|cag|gct|gaa|gac|gag|gct|gac|tac|tat|tgt|-3'

(Lm3BLo825) [RC]: 5'-gtc|ttc|ggc|ggt|ggt|acc|aaa|ctt|act|gtc|ctc|ggt|caa|cct|aag|g-
acacaggtgag-3'

(Lm3BSh832) [RC]: 5'-c|ggt|caa|cct|aag|gacacaggtgag-3'

(Lm3vg817): 5'-gac|gag|gct|gac|tac|tat|tgt|-
|<4>|<5>|<4>|<2>|<4>|tct|<4>|<4>|<4>|<4>|-
Gtc|ttc|ggc|ggt|ggt|acc|aaa|ctt|ac-3'

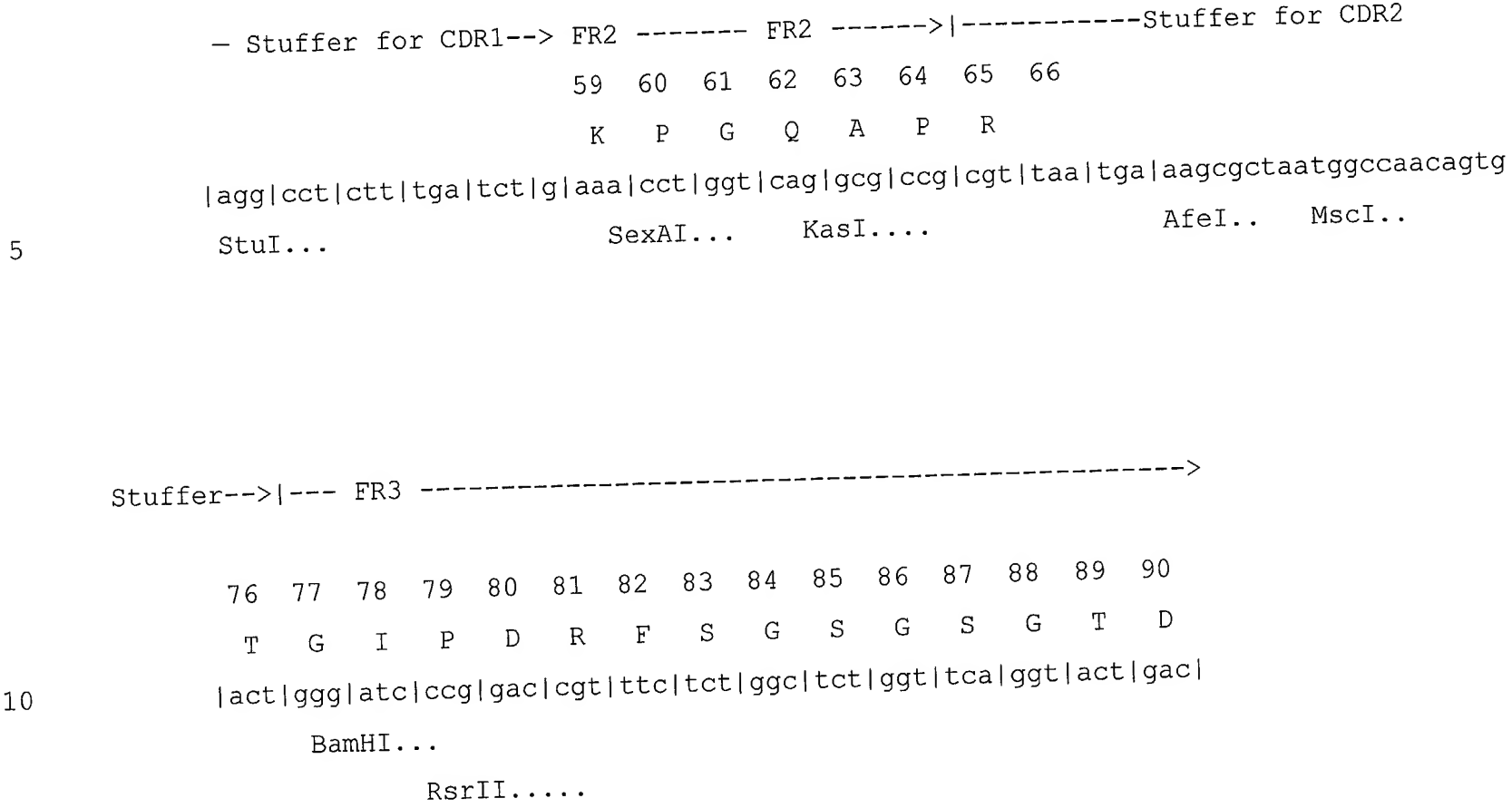
(Lm3vg817-10): 5'- gac|gag|gct|gac|tac|tat|tgt|-
|<5>|agc|tat|<1>|<5>|tct|<5>|<1>|<4>|gtc|ttc|ggc|ggt|ggt|-
|acc|aaa|ctt|ac-3'

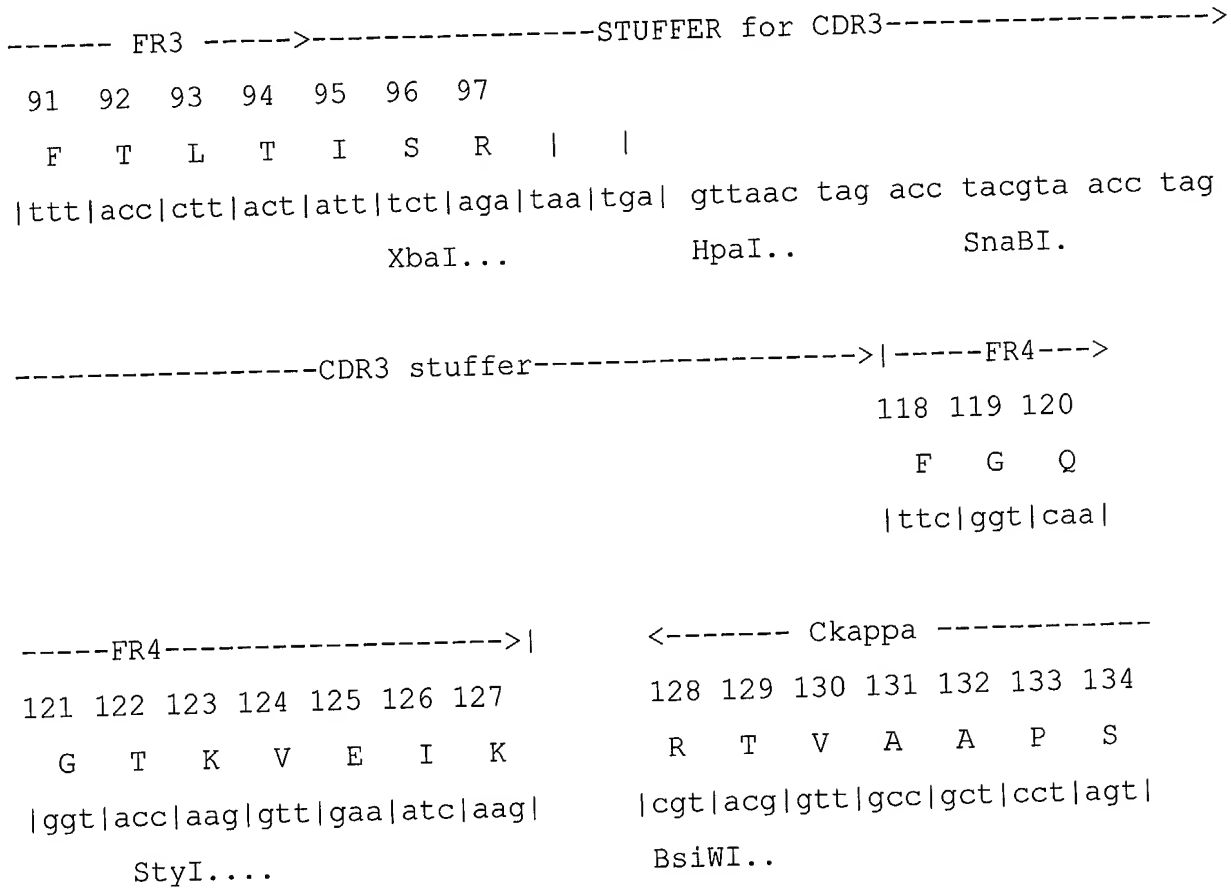
Table 10: A27:JH1 Kappa light chain gene with stuffers in place of CDRs

Each stuffer contains at least one stop codon and a restriction site that will be unique within the diversity vector.

```
gaggacc attgggcccc ctccgagact ctcgagcgca
  Scab.....EcoO109I
          ApaI.
                              XhoI..

acgcaattaa tgtgagttag ctcactcatt aggcacccca ggctttacac tttatgcttc
      ..-35..          Plac                        ..-10.

cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacacagga aacagctatgac

catgatta cgccaagctt tggagccttt tttttggaga ttttcaac
          PflMI.......
            Hind3.
```

```
M13 III signal sequence (AA seq)---------------------------->
 1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
 M   K   K   L   L   F   A   I   P   L   V   V   P   F   Y
gtg aag aag ctc cta ttt gct atc ccg ctt gtc gtt ccg ttt tac

--Signal--> FR1------------------------------------------->
16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
 S   H   S   A   Q   S   V   L   T   Q   S   P   G   T   L
|agc|cat|agt|gca|caa|tcc|gtc|ctt|act|caa|tct|cct|ggc|act|ctt|
          ApaLI...

----- FR1 --------------------------------->|-------Stuffer->
 31  32  33  34  35  36  37  38  39  40  41  42  43
  S   L   S   P   G   E   R   A   T   L   S   |   |
|tcg|cta|agc|ccg|ggt|gaa|cgt|gct|acc|tta|agt|tag|taa|gct|ccc|
   EspI.....                           AflII...
         XmaI....
```

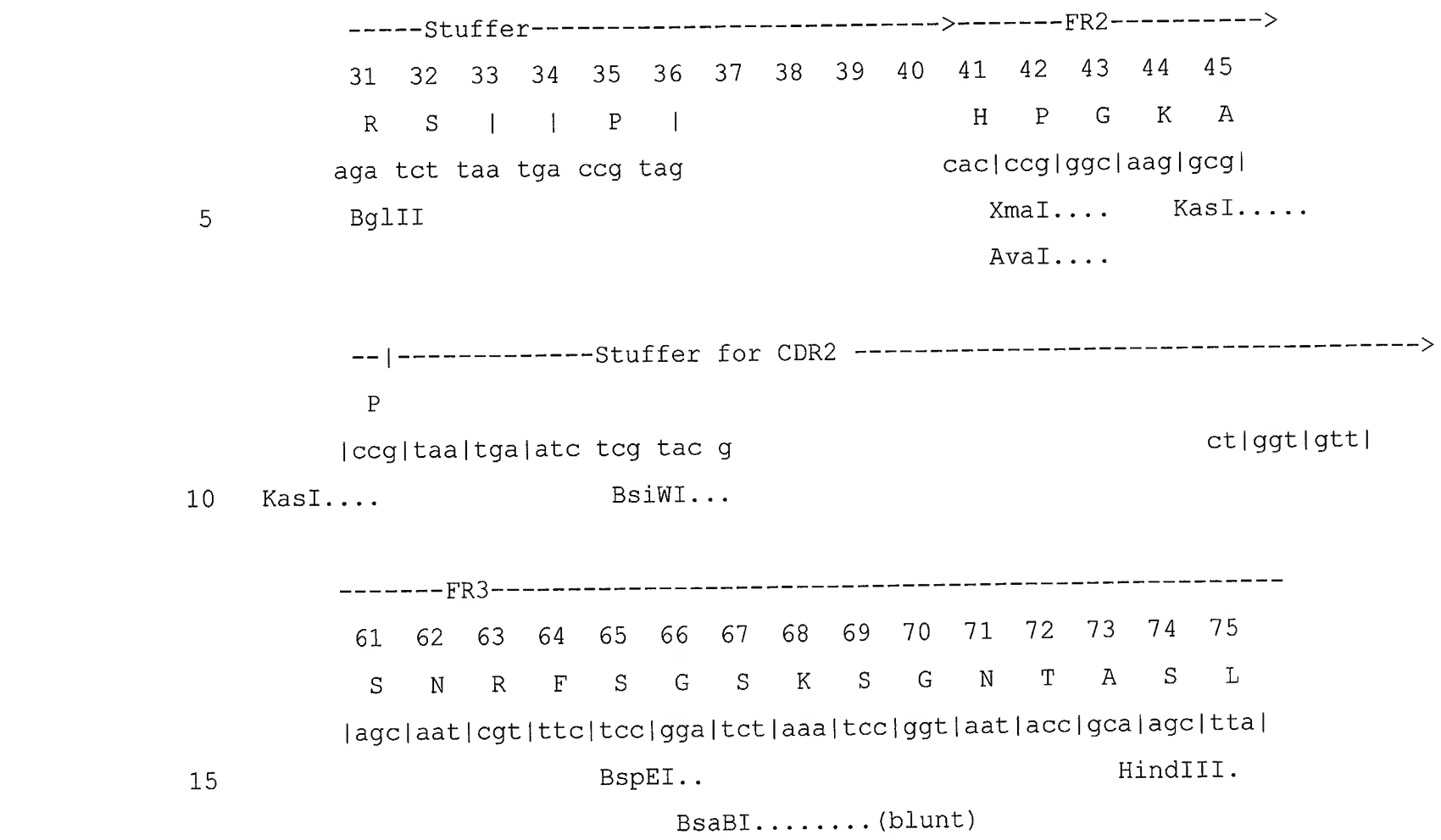

— Stuffer for CDR1--> FR2 ------- FR2 ------>|-----------Stuffer for CDR2
59 60 61 62 63 64 65 66
K P G Q A P R
|agg|cct|ctt|tga|tct|g|aaa|cct|ggt|cag|gcg|ccg|cgt|taa|tga|aagcgctaatggccaacagtg
StuI...
SexAI...
KasI....
AfeI..
MscI..
Stuffer-->|--- FR3 ---------->
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
T G I P D R F S G S G S G T D
|act|ggg|atc|ccg|gac|cgt|ttc|tct|ggc|tct|ggt|tca|ggt|act|gac|
BamHI...
RsrII.....


------ FR3 ----->----------STUFFER for CDR3---------->
91 92 93 94 95 96 97
F T L T I S R | |
|ttt|acc|ctt|act|att|tct|aga|taa|tga| gttaac tag acc tacgta acc tag
XbaI... HpaI.. SnaBI.
----------CDR3 stuffer---------->|-----FR4--->
118 119 120
F G Q
|ttc|ggt|caa|
-----FR4---------->|
121 122 123 124 125 126 127
G T K V E I K
|ggt|acc|aag|gtt|gaa|atc|aag|
StyI....
<------- Ckappa ----------
128 129 130 131 132 133 134
R T V A A P S
|cgt|acg|gtt|gcc|gct|cct|agt|
BsiWI..

```
135 136 137 138 139 140 141 142 143 144 145 146 147 148 149
 V   F   I   F   P   P   S   D   E   Q   L   K   S   G   T
|gtg|ttt|atc|ttt|cct|cct|tct|gac|gaa|caa|ttg|aag|tca|ggt|act|
                                    MfeI...

acgcatctctaa gcggccgc aacaggaggag
             NotI....
              EagI..
```

Table 11: 2a2:JH2 Human lambda-chain gene with stuffers in place of CDRs

```
gaggaccatt gggcccc   ttactccgtgac
Scab..... Eco0109I
          ApaI..

          ----------FR1---------------------------------------->
                1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
           S   A   Q   S   A   L   T   Q   P   A   S   V   S   G   S   P   G
          agt|gca|caa|tcc|gct|ctc|act|cag|cct|gct|agc|gtt|tcc|ggg|tca|cct|ggt|
           ApaLI...                          NheI...          BstEII...
                                                                SexAI....

------FR1----------------> |-----stuffer for CDR1---------
 16  17  18  19  20  21  22  23
  Q   S   I   T   I   S   C   T
|caa|agt|atc|act|att|tct|tgt|aca|tct tag tga ctc
                            BsrGI..
```


-----Stuffer----------->-------FR2---------->
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
R S | | P | H P G K A
aga tct taa tga ccg tag cac|ccg|ggc|aag|gcg|
BglII XmaI.... KasI.....
AvaI....
--|----------Stuffer for CDR2 ---------->
P
|ccg|taa|tga|atc tcg tac g ct|ggt|gtt|
KasI.... BsiWI...
-------FR3----------
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
S N R F S G S K S G N T A S L
|agc|aat|cgt|ttc|tcc|gga|tct|aaa|tcc|ggt|aat|acc|gca|agc|tta|
BspEI.. HindIII.
BsaBI.....(blunt)

```
-------FR3------------>|--Stuffer for CDR3----------------->|
 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
  T   I   S   G   L   Q
|act|atc|tct|ggt|ctg|cag|gtt ctg tag ttc caattg ctt tag tga ccc
                  PstI...                 MfeI..

-----Stuffer------------------------------>|---FR4---------
                                                103 104 105
                                                 G   G   G
                                               |ggc|ggt|ggt|
                                                         KpnI...

-------FR4-------------->
 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
  T   K   L   T   V   L   G   Q   P   K   A   A   P   S   V
|acc|aaa|ctt|act|gtc|ctc|ggt|caa|cct|aag|gct|gct|cct|tcc|gtt|
KpnI...                     HincII..
                                  Bsu36I...
```

```
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
 T   L   F   P   P   S   S   E   E   L   Q   A   N   K   A
|act|ctc|ttc|cct|cct|agt|tct|gaa|gag|ctt|caa|gct|aac|aag|gct|
                                SapI.....

136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
 T   L   V   C   L   I   S   D   F   Y   P   G   A   V   T
|act|ctt|gtt|tgc|ttg|atc|agt|gac|ttt|tat|cct|ggt|gct|gtt|act|
                    BclI....
```